

Pathogenic prion protein fragment (PrP_{106–126}) promotes human immunodeficiency virus type-1 infection in peripheral blood monocyte-derived macrophages

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ABSTRACT

Transfusion of blood and blood products contaminated with the pathogenic form of prion protein PrP^{Sc}, thought to be the causative agent of variant Creutzfeldt–Jakob disease (vCJD), may result in serious consequences in recipients with a compromised immune system, for example, as seen in HIV-1 infection. In the present study, we demonstrate that treatment of peripheral blood monocyte-derived macrophages (MDM) with PrP_{106–126}, a synthetic domain of PrP^{Sc} that has intrinsic functional activities related to the full-length protein, markedly increased their susceptibility to HIV-1 infection, induced cytokine secretion, and enhanced their migratory behavior in response to N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP). Live-cell imaging of MDM cultured in the presence of PrP_{106–126} showed large cell clusters indicative of cellular activation. Tyrosine kinase inhibitor STI-571, protein kinase C inhibitor K252B, and cyclin-dependent kinase inhibitor olomoucine attenuated PrP_{106–126}-induced altered MDM functions. These findings delineate a previously undefined functional role of PrP_{106–126}-mediated host cell response in promoting HIV-1 pathogenesis.

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Introduction

Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative disorders in both animals and humans (Beisel and Morens, 2004). In most TSE-affected individuals, abnormal prion protein (PrP^{Sc}) accumulates in the brain, where it is thought to play both direct and indirect roles in the pathogenesis of prion diseases. PrP is also found in a wide variety of peripheral tissues, including mononuclear blood cells (Cashman et al., 1990). With the identification of the variant vCJD, there were concerns that PrP^{Sc} might be inadvertently transmissible via iatrogenic routes, including use of blood and blood products (Sutton et al., 2006; McDonnell and Burke, 2003; Peden et al., 2005; O'Dowd, 2013); these concerns have since been borne out. Thus, individuals with a compromised immune system, especially HIV-1-infected patients, may be at a higher risk for disease complications.

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In the present study, we have used the synthetic peptide PrP_{106–126} as a model to evaluate the relationship between HIV-1 and PrP^{Sc} and to define a possible role of PrP^{Sc} in the pathogenesis of HIV-1 infection. We have shown that exposure of human MDM to the PrP_{106–126} activated MDM, enhanced their susceptibility to HIV-1 infection, and promoted their migratory activity. Since the transmission of TSE infectivity, if present in blood or blood products testing negative for HIV and other pathogens, could constitute a serious health hazard for the recipients, our studies may provide useful insights into the pathogenesis of TSE-HIV-1 co-infection.

Results and discussion

Transfusion of tainted blood or blood products has been demonstrated to be a mode of transmission of the vCJD to recipients (Llewellyn et al., 2004). Pathogenic consequences of TSE transmission to HIV-1-infected patients whose immune system is already severely compromised may be detrimental to the recipients disease status. Although there are no reports yet documenting increased incidence of the vCJD in general HIV-infected patients, addressing this previously undefined subject will advance our understanding of HIV-1 pathogenesis in TSE-HIV-1 co-infection.

Treatment of MDM with PrP_{106–126} increased their susceptibility to HIV-1 infection in a dose-dependent manner (Fig. 1A). PrP_{106–126} promoted HIV replication in MDM when treated 24 h before, at the time of, or even 2 h after infection (Fig. 1B). Consistent with the enhancement of viral replication, PrP_{106–126} treatment produced a marked increase in HIV-associated cytopathic effects in MDM as evident by the formation of multinucleated giant cells (Fig. 1C).

Live-cell imaging of MDM cultured in the presence of PrP_{106–126} for 72 h revealed marked aggregation of cells in discrete clusters, while similar incubations of untreated MDM or those treated with control ScrPrP peptide did not promote the formation of multicellular aggregates (Fig. 1D). Such homotypic aggregation of cells eventually resulted in the formation of increased numbers of

multinucleated giant cells after HIV-1 infection (as shown in Fig. 1C). These data suggest that treatment of MDM with PrP_{106–126} may be sufficient to initiate cell aggregation, but productive HIV infection is required for syncytium formation. Thus, our results provide strong support for recognizing a critical role of PrP-induced cellular activation in promoting HIV infection.

The cellular and molecular neuropathology of TSE, Parkinson's disease, and Alzheimer's disease suggest proinflammatory cytokines as possible pathogenic mediators in neurodegenerative disease (Bacot et al., 2003; McGeer et al., 1993; Kim et al., 1999; Williams et al., 1994, 1997). We examined for PrP_{106–126} induction of cytokine secretion by MDM. Consistent with previous reports (Lu et al., 2012; Fabrizi et al., 2001), we found significantly elevated levels of IL-1 β , IL-6 and TNF α in

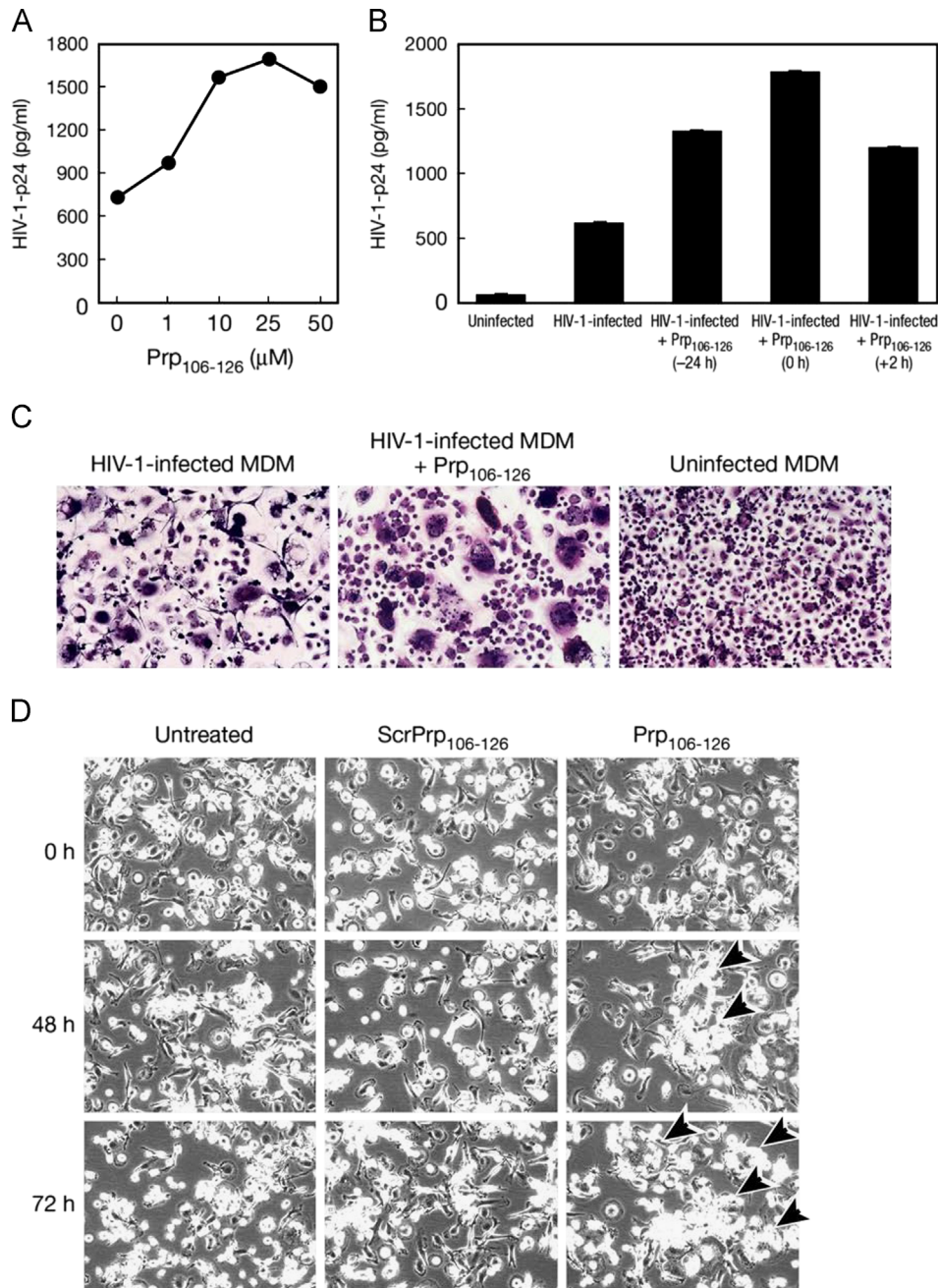


Fig. 1. PrP_{106–126} activation promotes HIV-1 infection of primary human MDM. Panel A. MDM treated with various concentrations of PrP_{106–126} 24 h prior to inoculation with HIV-1. Culture fluid was harvested 7 days after infection and assayed for HIV-1-p24. Panel B. HIV-1 p24 levels measured on day 7 in the culture supernatants from HIV-1-infected MDM treated with PrP_{106–126} (25 μM) 24 h before, at the time of, and 2 h after infection. Panel C. HIV-induced cytopathic effects in monocytes infected with HIV in the absence or the presence of 25 μM PrP. Panel D. PrP_{106–126}-induced activation of primary human MDM by live-cell imaging for the formation of cell aggregates (arrowheads).

the culture supernatants of MDM cultured for 24 h in the presence of PrP_{106–126}. These cytokines were nearly undetectable in culture supernatants from the untreated MDM or those treated with the control ScrPrP peptide (Fig. 2).

Macrophages are widely circulating cells of the immune system with the ability to migrate across the endothelium (Westhorpe et al., 2009; Rezaie and Al-Sarraj, 2007). They are known targets for HIV-1 infection, and are thought to be involved in the transmission of TSE infection. To examine the ability of PrP_{106–126} to promote the migratory activity of macrophages, we performed chemotactic assays on uninfected and HIV-1-infected MDM cultured in the absence or presence of PrP_{106–126}, using fMLP as a chemoattractant. Treatment of the monocytes with 25 μ M PrP_{106–126} for 24 h significantly increased their migratory response to fMLP. This increase in migratory behavior persisted in MDM in which PrP_{106–126} activation promoted the migration of both uninfected and HIV-1-infected MDM (Fig. 3A) in response to fMLP. PrP_{106–126} has been reported to induce the migration of macrophages (Zhou et al., 2009). Our results confirm these findings and demonstrate that PrP_{106–126} have a potent chemotactic effect on MDM (Fig. 3B). To evaluate PrP_{106–126} peptide specificity in activating MDM, we used the chemokine polypeptide LD78b as an additional control. As shown in Fig. 3, even though LD78b induced the migration of MDM similar to PrP_{106–126} when used as a chemoattractant, it suppressed HIV replication in infected cells (Fig. 3C). Taken together, these findings indicate that PrP^{Sc} may play an important role in modulating some of the immunological functions ascribed to specific cells in immune disorders.

The mechanism by which our peptide fragment induces MDM activation and causes altered biological functions might be due to PrP_{106–126}-mediated signal transduction. To test this hypothesis,

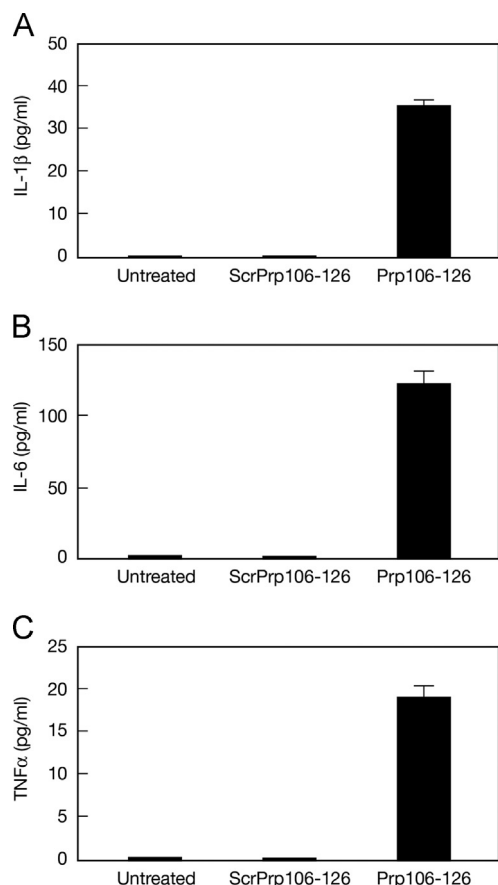


Fig. 2. PrP_{106–126} activation promotes inflammatory cytokine production by primary human MDM. Panel A: IL-1 β ; panel B: IL-6; panel C: TNF α .

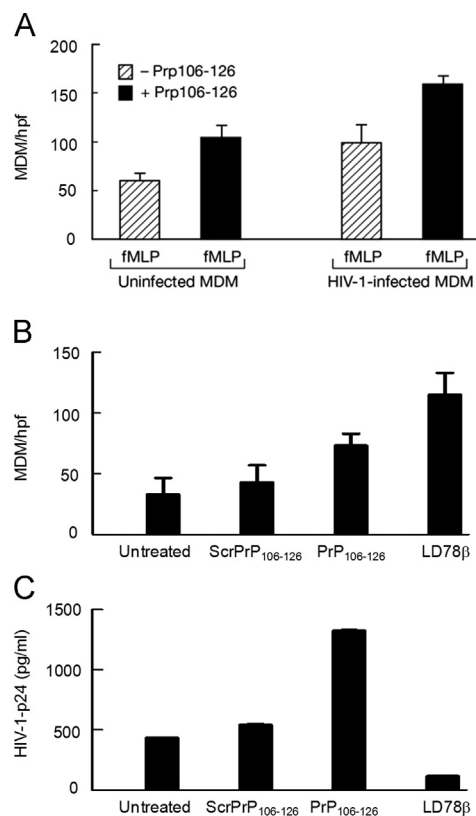


Fig. 3. Panel A. PrP_{106–126}-induced chemotactic activity of uninfected and HIV-1-infected MDM in response to fMLP. Panel B. Chemotactic response of MDM in response to PrP_{106–126}. The number of migrated cells attached to the underside of the filter was determined by high power microscope field (hpf) in triplicate for each well, and experiments were conducted in groups of three for each experimental point as described in the section “Methods”. Panel C. Effect of PrP_{106–126} and LD78b on HIV replication in MDM. MDM were cultured in the absence or presence of 25 μ M PrP_{106–126} or 100 ng/ml LD78 β for 7 days at 37 °C. Culture fluid was harvested 7 days after infection and assayed for HIV-1-p24. Error bars indicate standard deviations $p < 0.05$.

MDM were incubated with PrP_{106–126} in the presence of various concentrations of tyrosine kinase inhibitor STI-571, protein kinase C inhibitor K252B, and cyclin-dependent kinase inhibitor olomoucine, and then infected with HIV-1. Cell-free virus was quantified by an HIV-1-p24 ELISA on day 7, and cell migration was evaluated by chemotaxis assay. As shown in Fig. 4, all inhibitors substantially attenuated HIV-1 replication, even in cells not treated with PrP_{106–126}; however, the PrP_{106–126}-induced increase in HIV-1 replication was markedly suppressed (Panels A–C). Similarly, these inhibitors inhibited the migration of both untreated and PrP_{106–126}-treated MDM in response to fMLP; however, migratory activity of PrP-activated MDM in response to fMLP was much lower (Panels D–F). Although the downstream pathways through which PrP_{106–126} stimulates various kinases in MDM remain to be elucidated, our studies using PrP_{106–126} as a model for PrP^{Sc} can provide a basis for understanding the functional role of PrP^{Sc} in cellular activation resulting in the progression of HIV-1 infection. PrP_{106–126} activation of MDM is consistent with our previous observations of PrP-induced stimulation of proinflammatory responses in human monocyte-derived dendritic cells (Bacot et al., 2003). Because of the current absence of a sensitive diagnostic assay, PrP^{Sc} (and by extension, TSE infectivity) is not yet detectable in blood, and concerns remain about transmission of this pathogenic agent through blood transfusion (O'Dowd, 2013; Farrugia, 2002; Hunter et al., 2002; Gregori et al., 2011; Houston et al., 2008). In summary, the findings of the present study provide strong evidence for a role of PrP^{Sc} in promoting the pathogenesis of HIV-1 infection.

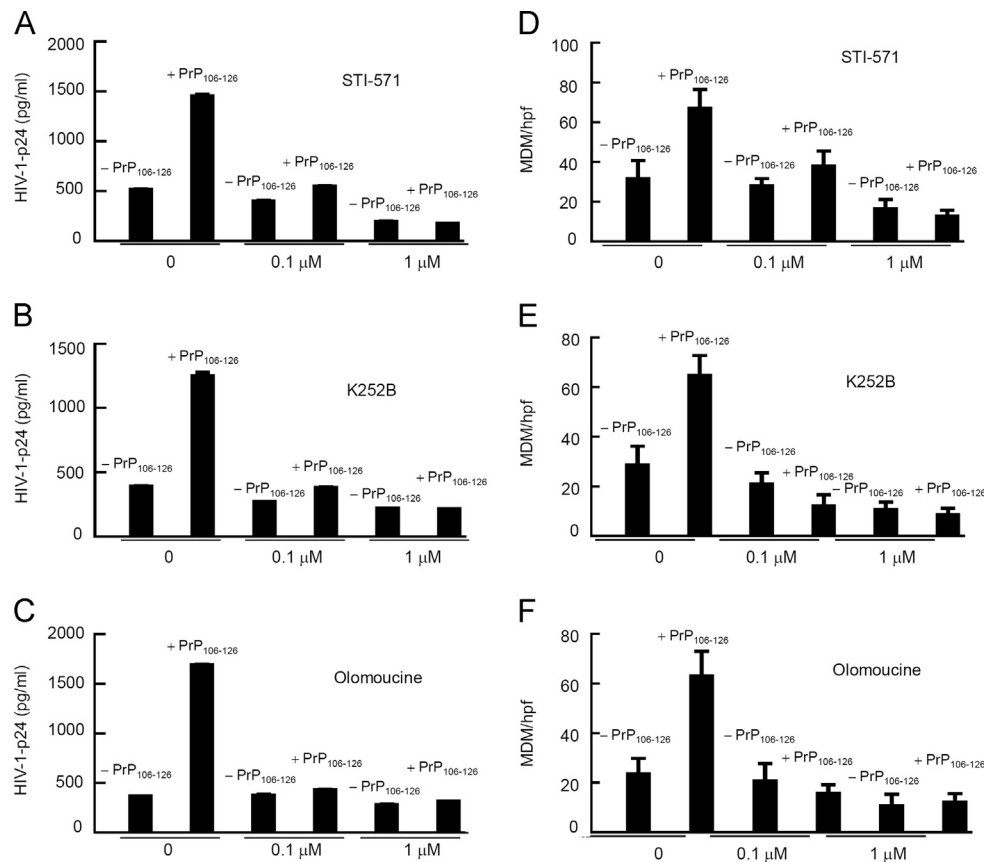


Fig. 4. Inhibition of PrP₁₀₆₋₁₂₆-induced HIV-1 replication in MDM by protein kinase inhibitors. Panels A–C. MDM were pre-treated with the indicated protein kinase inhibitors (0–1 μM) 30 min prior to HIV-1 infection. Culture fluids were harvested 7 days after infection, and cell-free HIV-1-p24 was determined by ELISA. Panels D–F. Inhibition of PrP₁₀₆₋₁₂₆-induced migration of MDMs by protein kinase inhibitors. MDMs were pre-treated for 30 min with the indicated protein kinase inhibitors (0–1 μM) and then incubated in the presence of 25 μM PrP₁₀₆₋₁₂₆ for 24 h at 37 °C before being assessed for chemotactic potential as described in the section “Methods”. The number of cells attached to the underside of the filter was determined in triplicate for each well, and experiments were conducted in groups of three for each experimental point. Error bars indicate standard deviations. $p < 0.05$.

Methods

Human MDMs were generated from normal donors (Wahl et al., 1984) infected with HIV-1BaL as described, (Devadas and Dhawan, 2006) and maintained in DMEM supplemented with 10% FBS, 20 μg/ml gentamicin, and 1000 U/ml macrophage colony stimulation factor (M-CSF). Culture media was replenished with fresh media every other day. HIV replication was quantified by measuring cell-free HIV-p24 in culture supernatants using the NEN/Dupont ELISA analysis kit (Perkin Elmer Life Sciences, Inc., Boston, MA) according to the manufacturer's instructions as previously described (Devadas and Dhawan, 2006).

A synthetic peptide corresponding to amino acid residues 106–126 of the PrP^{Sc} (PrP₁₀₆₋₁₂₆), known to contain biological activity, (Forloni et al., 1993; Selvaggin et al., 1993) was synthesized in-house as described previously (Bacot et al., 2003). Biological activity of the PrP₁₀₆₋₁₂₆ peptide was tested by calcium mobilization analysis (Bacot et al., 2003). A scrambled prion peptide (ScrPrP), purchased from Sigma-Aldrich, St. Louis, MO, was used as a negative control. In-house peptides were extracted three times using a solution of 50:50 acetonitrile (Baxter, Muskegon, MI):H₂O (v/v) and dissolved in water at a concentration of 1 mM.

MDM were cultured in the absence or presence of ScrPrP₁₀₆₋₁₂₆ (control prion peptide), or PrP₁₀₆₋₁₂₆ (functional prion peptide) at 37 °C for up to 72 h. Cell culture morphology was monitored with parallel inverted microscopes equipped for phase-contrast microscopy (Zeiss Axiovert 25) with 37 °C humidified chambers at 10% CO₂ (Precision Plastics) using 5X Zeiss A Plan, 0.12N.A. objectives. Images were collected with digital cameras (Infinity2; Luminera) at 10 min

intervals using Infinity2 software, and the contrast and brightness of images were adjusted in parallel using Photoshop software.

Supernatants of media collected from MDM cultured for 24 h at 37 °C in the absence or presence of PrP₁₀₆₋₁₂₆ or ScrPrP were analyzed for IL-1β, IL-6, and TNFα. Cytokine levels were determined by using the ELISA kits from R&D Systems according to the manufacturer's instructions.

Cell migration assays were conducted in 48-well microchemotaxis chambers (Neuroprobe Inc., Gaithersburg, MD) (Zhou et al., 2009). Briefly, untreated and PrP₁₀₆₋₁₂₆-treated MDM were placed in the upper chambers. FMLP, used as a chemoattractant at a concentration of 10⁻⁸ M in a volume of 26 μl of RPMI containing 1 mg/ml BSA, was placed in the lower chambers. A polycarbonate polyvinylpyrrolidone-free filter with a pore size of 8 μm (Neuroprobe) was then placed over the lower chambers, and the unit was assembled. The chambers were incubated for 3 h at 37 °C. The filters were then carefully removed, fixed, and stained with Diff-Quick stain (Fisher Scientific). Cells on the upper side of the filter were removed, and those attached to the underside of the filter were counted using a high power microscope field (1.4 mm²) in triplicates for each well.

Authorship

Contribution: S.D.: designed the study, performed experiments, and wrote the paper; S.B. performed experiments; S.D., S.B. G.F. and K.M.Y. interpreted data.

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